Author's response to reviews

Title: Quantification and Physiological Significance of the Rightward Shift of the V-slope during Incremental Cardiopulmonary Exercise Testing

Authors:
Hirotaka Nishijima (hnishiji2002@jcom.home.ne.jp)
Kazuo Kondo (byx05256@nifty.com)
Kazuya Yonezawa (kyonezaw@hnh.hosp.go.jp)
Hiroki Hashimoto (hashi.20101231@gmail.com)
Masayuki Sakurai (sakura@hokko.or.jp)

Version: 2 Date: 16 Mar 2017

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The authors' response letter has been also included as a supplementary file.

Response to reviewers, Mar 2017 BMC Sports:

SSMR-D-17-00004

Quantification and Physiological Significance of the Rightward Shift of the V-slope during Incremental Cardiopulmonary Exercise Testing Hirotaka Nishijima, MD, PhD; Kazuo Kondo; Kazuya Yonezawa, MD, PhD; Hiroki Hashimoto; Masayuki Sakurai, MD, PhD BMC Sports Science, Medicine and Rehabilitation

Reviewer reports:
Reviewer 1: The authors present an interesting paper examining the VAT and associated right shift in a clinical population. The paper is very well written, there is a clear research question, the methods provide a comprehensive approach to data handling, the discussion interprets the those results and the findings may have some applied, clinical applications. Importantly the research
meets the criteria of the Journal; the study is scientifically valid, with a sound research question and suitable methods.

The only area that requires attention is some of the statistical analyses. You disclose that your right shift data are not normally distributed, and this variable is involved in most of your analyses. However, even though the data is non-normal you apply parametric testing throughout (Pearson's correlation, students t-test, one-way ANOVA) with the right shift data. Those analyses should be rerun with a non-parametric equivalent to reflect the properties of the data. I doubt this will make any material difference to the findings or interpretation, but it is needed to be statistically correct.

Response

Thank you very much for highly evaluating our research.

We quite agree with you that analyses using non-parametric should also be applied, as the data distribution is non-normal. The methods and results are described in the methods (Lines 241-244) and results (Lines 299-300) section, respectively. The detailed statistical results are summarized in an additional file (Result S1).

Reviewer 2: General feedback and major comments This is an original investigation on the rightward shift of the V slope during cardiopulmonary exercise testing in a cohort of patients with cardiac disease. It was found that the quantification of the rightward shift offers a more objective measure of physical capacity, as it appears to correlate rather well with the ventilatory anaerobic threshold. This RtShift is thought to be mainly caused by the capacity of muscle fibres to store CO2 in the early stages of exercise. Most parts of the manuscript are well presented, it is a little unclear what the addition of the "substudy" adds to the manuscript - I would be tempted to either provide a little more detail there or to leave it out altogether. Details about the discussed 6-min exercise bouts are also unclear.

For most parts, the study is carefully presented; especially the figures and supplementary material are very clear and well made. The main criticism I have is that there is a mix of information in the subsections, for example the methods section contains a lot of results, and
various aspects of the study are discussed in the wrong sections. Some arguments need to be reworded as they are a little unclear, see specific comments (even though on the whole the English language is excellent given that this is a second language to the author team).

Response:

Thank you very much for reading the manuscript thoroughly and critically, and giving us very constructive comments.

I) Concerning the substudy.

The substudy was performed because we were not sure how a ramp change of exercise protocol affected the size of RtShift. If the RtShift is dependent on the recruited muscle mass, then a steeper ramp may result in a greater RtShift. We added this reason for doing this substudy on Lines 375-378.

II) The six-min exercise bouts.

The six-min exercise bouts were performed by a normal male volunteer (one of the co-authors). The sole purpose of the experiment was to show the phenomenon of CO2 storage in the framework of V-slope. This phenomenon has been repeatedly demonstrated in the literature we cited. However, it has always been depicted graphically, with the elapsed time (s or min) on the x-axis and VCO2 on the y-axis. We instead wanted to show this phenomenon in the framework of the V-slope, because that is the aspect of the phenomenon that we are dealing with in this paper. This was added on Lines 147-152 in the Methods section and on Lines 257-260 in the Results section.

III) Inappropriate placement of certain aspects of study such as Methods, Results and Discussion.

These were corrected as addressed in the response to each specific comment. Due to content placement changes, the figure numbers and/or reference numbers were changed accordingly.
Specific comments

Abstract

1. Line 60/61: it is unclear what you mean with "vertical" vector for VAT. For RtShift I can see that it was determined based on the horizontal distance from the line formed by R=1, but as VAT is determined visually I don't quite see what you mean with the notion of a vector in this context.

We reasoned as follows: On V-slope, the RtShift is the horizontal vector; the predominant force of VAT is vertical (although tilted at 45 degrees). However, as you suggest, this might not help and indeed may be unnecessary in the understanding of RtShift. As such, we have decided to omit the explanations using the vector altogether (Line 60).

2. Line 76: "unique" may not be the appropriate word here. Do you want to say that it is a further advantage of RtShift?

We deleted the word “unique.”

Methods

3. Line 110: delete "consecutive" (also in abstract)

We deleted the word “consecutive.”

4. Table 1: how were LVEF and LVDd determined? Include details of these procedures (device used for example)

We described the device and the procedures as in Lines 227-230.
5. Line 133: what was the start load? zero watts?

For the ramp protocol of 5 W\textsuperscript{-min-1}, 10 W\textsuperscript{-min-1}, and 15 W\textsuperscript{-min-1}, the warm-up load (W) was 0, 10, and 15, respectively; and the ramp start load (W) was 0, 10 and 15, respectively. This was added in Lines 136-138.

6. Line 134: what was the warmup intensity? This is crucial as it appears that the warm-up data had been included in the analysis

The warm-up intensity was described in Response #5. As described in the original manuscript, for the mathematical derivation of RtShift, all data points that were equal to or below R=1 were used throughout the resting and exercise periods. This included the warm-up exercise period (Lines 191-193). This segment has not been altered from the original manuscript.

7. Lines 159-161, 163-169: there are some results reported here (whenever p values are reported) - these should be given in the results section.

This section was divided into the methods and the results sections (Methods: Lines 164-169; Results: Lines 285-295).

8. Line 168-169: what are the limits of agreement analyses based on? Were the VATs determined by two people independently and the variation between VATs assessed? Or what do the limits of agreement refer to?

The VAT reliability study was performed in 2009 by using CPX records of 100 cardiac patients. Two assessors participated in this blinded reading study of VAT with a controller assigned, who gave two assessors coded CPX records that had been randomly ordered. Reading was performed twice, three weeks apart. LOAs for VAT for two assessors were each 144 and 210 ml/min VO2, respectively. The LOA calculated from the average of two readings of two assessors was 170 ml/min VO2. The details have been added to the text (Lines 174-175). These results were presented at the 2009 Japanese Cardiac Rehabilitation annual meeting. The value of 170 was close to the value of 200 ml/min, cited by J. Myers (a multicenter evaluation of VAT, J Cardiac Failure 2010;16;76-83), which has been cited by our Medicine paper (Reference #10).
9. Line 177: this is confusing - In my view, in figure 1, VO2 at "c" equals to VCO2 at "c"... Please clarify this.

I believe you mean VO2 at “a”… instead of “c”…. The point “c” is only secondary, used for a possible rest RtShift correction.

In principle, RtShift is calculated as the distance from “a” to “b” (b minus a). However, in practice, it is simply calculated using the x, y values (VO2 minus VCO2) of the single data point “b,” because of the regular square nature of the conventional V-slope graph. The text was modified so as to follow this reasoning (Lines 184-185).

10. Line 181: "vertical component": this is confusing - there is a horizontal component in both A and B (VO2 increases in both cases as VCO2 increases)

This was addressed in Response #1. The vertical component or vector explanation was deleted and the sentences were modified (Lines 187-189)

11. Line 184-185: this is results section information

The information was moved to the results section as in Lines 256-257.

12. Line 185-190: this is discussion section information

These lines were deleted, because they overlap the first paragraph of the discussion section.
13. Line 192: this must be described in methods section: with which participants was this performed? You mention a sub-study, but this was only done with different ramps (?)

This was addressed in Response #II.

14. Line 197: again, what power output for the warm-up? so warm-up values were included in analysis?? and also resting values? how many minutes worth of the resting data were included?

This was addressed in Response #5. For resting data, we collected one minute.

15. Line 205: but you also refer to a third method (visual) - this should be mentioned here.

This method was also mentioned together with other two methods as in Line 201. The terminology was also changed as follows; the visual method was termed the first method, curve fitting as the second, and simple averaging as the third method (Lines 202, 207, 215).

16. Line 216: more detail is needed on how you quantified this visually.

The detail was supplied as in Lines 202-207.

17. Line 223: which values were used to determine this? only the ones below VAT? or all values?

All values were used. As described in this reference #10 (Medicine), the initial time delay was visually removed from analysis according to Hansen et al (Am J Cardiol 1987; 59669-674). Since our study was submaximal, most of the data points used for analysis were below VAT.
18. Line 225-229: this is a repetition from lines 140-142

This section was deleted.

19. Line 230: I don't think this was mentioned in the methods section (also bearing in mind that the statistics itself are part of the methods section)...

True, it had not been mentioned. Therefore, the sentence was corrected as in Line 232.

20. Line 234-237: this should be mentioned in the discussion section.

It was moved to the discussion section as in Line 379-385.

Results

21. Table 2: give more detail on the IQR (report lower and upper quartile separately)

The lower and upper quartiles were added to Table 2.

22. Line 258: "congruent": better: "correlated well"?

The sentence was changed as suggested as in Line 265-266.

23. Line 261: "failure rate": how was this defined?

With the second method, the regression curve had to be a convex one within the data range. If this feature was not obtained, it was considered a failure. This was added in Lines 212-213 of Methods. In addition, in the Results section, a 10% failure rate was corrected to a more exact 11% (Line 269).
24. Line 269-271: this is methods information

It was moved to the Methods section (Lines 239-244).

25. Line 275: more detail needed (how was this assessed statistically - report in methods section), and improve sentence construction

The statistical method (multiple linear regression) was added as in Lines 239-241, and the sentence construction in the Results section was changed as in Lines 280-281. As described in the text, the use of beta-blockers was entered into the multiple linear regression as a dichotomous variable. In addition, the mean amount of RtShift between without and with beta-blocker was also not significantly different, either. (644 vs. 626 ml/min, p = 0.692).

26. Line 281: ANOVA: no need to repeat this here - this is methods section information

The word ANOVA was removed.

Discussion

27. Line 304-306: it is unclear what you are referring to here (not the graded exercise that you performed?)

I believe that most data on the energy source pattern is performed during steady state exercise protocols, and that the results show energy source change for fat occurs only after 20 minutes. I am speculating here that with ramp exercise too, there is no reason to believe that the energy change to fat occurs so early. The discussion was added as in Lines 325-327.
28. Line 309-310: I do not understand what is meant with this. Can this be reworded?

I apologize for a typing error in the original sentence of “This magnitude of change is too small compared with that of RtShift or S1”. S1 should have been S2. This was corrected and sentence construction was slightly changed as in Lines 321-333.

29. Line 317: S1: is this the "slope" of S1? make this clearer early on in the manuscript.

As you point out, S1 can denote the S1 slope itself or the slope of S1 (value). Therefore, tried to make it clear throughout the text. The S1 and S2 were also added to the list of abbreviations with explanations to clarify points above (Lines 404-406).

30. Line 329-330: make clearer why these results are contrary to the results of the current study. It is unclear how you relate the findings of the O2 deficit with the findings of your study.

As you point out, the two studies are not comparing the same index to CO2 storage; therefore, we cannot draw a conclusion here. We removed the above sentence and rewrote it as in Lines 353-355.

31. Line 338: the "phenomenon" is not necessarily aerobic, but it can be observed at submaximal ("aerobic") exercise intensity

Theoretically or ideally (Figure 1), the right-shifted S1 is completely aerobic; it is the stage before the appearance of VAT. However, in the actual calculation (or estimation) of RtShift of S1, we needed to use all of the data points from resting to exercise up to the point of R=1 (i.e., beyond VAT in cases with large RtShift). Therefore, I want to retain the original sentence “it is primarily an aerobic phenomenon” as it is.

32. Line 338: did you assess VO2peak and the correlations? in the substudy? I don't think there is any mention of this in the manuscript.
Although this study was a submaximal exercise study, we have in the additional file the previous results of the symptomatic maximal (Figure S1), which assesses VO2 peak and the correlations.

33. Line 341-343: as mentioned earlier, it is unclear what is meant with this (horizontal and vertical components).

This was addressed in Response #1.

34. Line 343-344: RtShift and VAT analysis: 10%. what is this based on? Make this clearer!

The mean VAT is 635 ml/min; the mean RtShift is 50.8, 42.0 and 33.9 ml/min, respectively, depending on the method of estimation (i.e., visual, quadratic or simple averaging). When based on our concept of RtShift (Figure 1), the true RtShift is probably closer to that of visual or quadratic regression (40-50; therefore, I wrote (about) 10% (50.8/635). Simple averaging obviously underestimates the level of RtShift in this sense, although as an index of RtShift, it is as good as the other two. However, I believe it is not too important to point these out. Therefore, I opted to delete this sentence Line 364).